Induced cough challenges in a single patient with COVID-19 showing an interplay between Nrf2, TRPA1 and TRPV1 agonists

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Short Title: Nutrients acting on cough challenges in COVID-19

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Abstract

Background: COVID-19 is commonly associated with impaired redox homeostasis responsible for reactive oxygen species acumulation. Antioxidants have been proposed as being effective in controlling COVID-19 symptoms. Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is the most effective antioxidant mechanism. TRPA1 (transient receptor potential ankyrin 1) is highly sensitive to oxidative stress and induces several COVID-19 symptoms. TRPA1 and TRPV1 (transient receptor potential vanillin 1) are potential candidates for COVID-19 symptoms.

Methods: Forty nine open labelled induced cough challenges were carried out on the same patient. Nutrients with various Nrf2 and TRPA1/TRPV1 agonist activity were used: broccoli seeds (potent Nrf2 agonist and mild TRPA1 agonist), berberine (Nrf2 only), black pepper, curcumin, ginger, green tea, resveratrol, Zinc (potent TRPA1 activity and variable Nrf2 agonist) and red pepper (potent TRPV1 agonist).

Results: Berberine and Zinc were not effective. All other nutrients except resveratrol were rapidly effective (1-10 minutes). The effect of green tea, curcumin + black pepper, ginger, resveratrol or red pepper disappeared in 1 to 4 hours. Broccoli had a longer duration of action (5-7 hours). The duration of the of the effect increased to around 10 hours when low doses of curcumin + black pepper or resveratrol were added to broccoli. Paracetamol low dose (its metabolite N-acetyl-p-benzoquinone imine is a TRPA1/TRPV1 agonist) increases the duration of action of combinations to over 14 hours.

Conclusions: A synergy between Nrf2 antioxidative effect and TRPA1 desensitization (and TRPV1) is proposed. Research should confirm this trial and find the mechanisms of synergy.

Key words: COVID-19, Nrf2, TRPA1, TRPV1, induced cough challenge, spices, sulforaphane

Introduction

Three stages of COVID-19 have been described: (i) a viral infection lasting for one to two weeks; (ii) a second phase characterized by an intertwined cytokine and oxidative stress storm, independent of infection; and (iii) a recovery phase that may last for some months. Many patients during the recovery phase suffer from persistent symptoms including cough.

A common denominator in all conditions associated with COVID-19 appears to be the impaired redox homeostasis responsible for reactive oxygen species (ROS) accumulation [1]. Many foods have antioxidant properties and many mechanisms may be involved. However, the activation of the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) the most potent antioxidant transcription factor may be of primary importance.[2,3] Differences in COVID-19 death rates among countries may in part associated with Nrf2 and Nrf2-interacting nutrients like fermented vegetables could reduce COVID-19 severity [4-6]. Many Nrf2-interacting nutrients such as spices are TRPA1 (transient receptor potential ankyrin 1) and/or TRPV1 (transient receptor potential vanillin 1) agonists [7]. TRPA1, an excitatory ion channel, plays a pivotal role in augmenting sensory or vagal nerve discharges, evoking several COVID-19 symptoms [7]. It is highly sensitive to oxidative stress. In COVID-19, rapid desensitization of TRAP1 and/or TRPV1 may reduce symptom severity [8] (and paper submitted and available online [9]).

The present report consists of the self-description of a COVID-19 case where the patient – who is also the author - describes induced cough challenges carried out during the recovery phase. The patient had moderate COVID symptoms during the first and second phases of COVID-19 (paper submitted and available online [8]). The patient presented persisting cough and nasal obstruction up to 65 days after the onset of COVID-19 symptoms. The challenges were carried out from 10 to 50 days after the cytokine storm with nutrients interacting with Nrf2, TRPA1 and TRPV1 aiming at differentiating time course of the effects and putative mechanisms.

1. Methods

1.1. Compounds tested

Broccoli was chosen as it contains 10% glucoraphanin, a precursor of sulforaphane the most potent Nrf2 natural activator [10-16]. Broccoli seed capsules containing 10% glucoraphanin (broccoli) were shown to be effective in 3 patients during the first two phases of COVID-19 (paper submitted and available online [8]). However, it is difficult to deliver sulforaphane in an enriched and stable form for human consumption [17]. Thus, glucoraphanin, the precursor of sulforaphane, is administered orally with myrosinase, the enzyme that transforms glucoraphanin into sulforaphane.

All nutrients tested have a variable Nfr2 agonist activity. Although the specificity of the different agonists is not clearcut it can be considered that curcuma and black pepper [18], ginger, green tea [19,20], quercetin and the plant polyphenol resveratrol [21] are all TRPA1 agonists, whereas berberine does not appear to have such an effect. Capsaicin from red pepper is the agonist of TRPV1 but it also has some TRPA1 activity. Zinc acts on Nrf2 [22] and TRPA1 [23].

Paracetamol increased the duration of efficacy (but not the efficacy) of broccoli treatment in three clinical cases (paper submitted and available online [8]). The electrophilic metabolites N-acetyl-p-benzoquinone imine (NAPQI, hepatotoxic metabolite) and p-benzoquinone, but not paracetamol itself, activate TRPA1 [24] and TRPV1 [25]. NAPQI also directly activates Nrf2 [26], and benzoquinone desensitizes TRPA1 [27]. The physiological and toxicological responses of paracetamol form a continuum coordinated by the Wnt and Nrf2 pathways. Therapeutic doses produce reactive ROS and NAPQI in the cytoplasm but result in little permanent damage [28]. The compounds used in the study are listed in Table 1.

Table 1: Compounds used in the study

Nutrient	Origin	Composition as indicated on the label and recommended daily dose	N challenges	Dose
Broccoli	Pileje, Paris, France	Broccoli seeds 300 mg Glucoraphanin 10 %	9+11	150 mg 300 mg
Ginger	Arkopharma, Carros, France	Gingerol 6.3 mg	1+2	6.3 mg 12.6 mg
Green tea (loose or bag)	Lipton, Antwerp, Belgium		3	3.0 g 4.5 g
Green tea capsules	Arkopharma, Carros, France	1750 mg green tea	1+2	1750 mg
Curcumin + black pepper		Curcuma powder 560 mg Curcumin 103 mg Black pepper 16 mg Piperine 15 mg	2+4	103+16 mg 51.5+7.5 mg
Quercetin	Dynaveo, St Gilles, France	500 mg, extracted from *Sophora japonica*, (HPLC tested)	1	500 mg
Resveratrol		600 mg, extracted from *Polygonum cuspidum* (HPLC tested)	3+5	600 mg 1200 mg
Berberine		1200 mg, extracted from *Berbera aristate* (HPLC tested)	2	1200 amg 2400 mg
Zinc	Rubozinc®, Lacbatal, France	Zinc gluconate, 15 mg of Zn	1+1	15 mg
Paracetamol	Sanofi	Up to 3 g per day	1+4*	250 mg 500 mg

N: administered alone or (+) in combination, * some compounds were given in combination

1.2. Induced cough challenges

1.2.1. Characteristics of the patient

The patient is allergic to grass pollen and cat, and has moderate to severe allergic rhinitis which is perfectly controlled by as needed medications. Further to the onset of COVID-19, he experienced mild to moderate nasal obstruction (VAS up to 6/10) and proposed a score for his symptoms according to the VAS for allergic rhinitis (from 0 to 10) [29-31]. The patient also suffers from mild intermittent asthma, especially when exposed to high levels of air pollution. He uses formoterol-budesonide low dose when needed and his maximal annual consumption is around 40 doses. He has a severe bronchial hyperreactivity. He used formoterol-budesonide low dose to reduce COVID-19 cough without benefit.

1.2.2. Design of the test

Many scores exist for cough [32,33]. A visual analogue scale (VAS) score for "spontaneous cough" was used. When the patient had tracheobronchial pruritus, a deep inspiration followed by a rapid expiration led to immediate "wheezing" (not similar to asthma but similar to exposure to high levels of air pollution) and cough that were scored. He used a score for induced cough during challenge. For spontaneous cough during challenge, he decided to count the number of coughs per episode (Table 3).

Table 2: Cough symptom score during challenge (from a paper submitted and available online [9])

Spontaneous cough	Score	Induced cough after fast and hard expiration	Score
≥5 coughs for one episode	3	persisting severe cough	9-10
2-4 coughs for one episode	2	>9 coughs or intolerable	8
1 cough for one episode	1	6-9 coughs	7
		3-6 coughs, mild	6
		2-3 coughs, severe	5
		2-3 coughs, mild	4
		1 cough	3
		Loud “wheezing”	2
		Some “wheezing”	1

1.2.3. Open labelled induced cough challenges

In order to test the induced cough challenge and the broccoli dose that could be effective, the patient performed three induced cough challenges with a dose of 300 mg of broccoli (daily recommended dose). Similar to the FEV_1, he took the deepest breath he could, exhaled as fast and as hard as possible and counted the number of coughs according to Table 2. He found that induced cough improved within 10 minutes after broccoli ingestion and nasal obstruction even more rapidly. He then selected this dose for the double-blind, placebo-controlled (DB-PC) challenges (reported in a paper submitted and available online [9]) and 10 pre-study open challenges. The DB-PC challenges (N=4 with broccoli and N=4 with lactose) confirmed the open challenges.

After more 60 days since the cytokine storm, the patient is still suffering from persistent cough and nasal obstruction, and needs the broccoli or other nutrient capsules when VAS for spontaneous cough >7/10. These episodes occur at around twice a day under treatment.

The patient performed a total of nine challenges carried out with broccoli capsules and 31 with Nrf2 and/or TRPA1-TRPV1 interacting nutrients (Table 1). Broccoli challenges were used to assess the reproducibility of the challenges and to test whether the tracheobronchial reactivity was similar over time since improvement of the disease was not predictable.

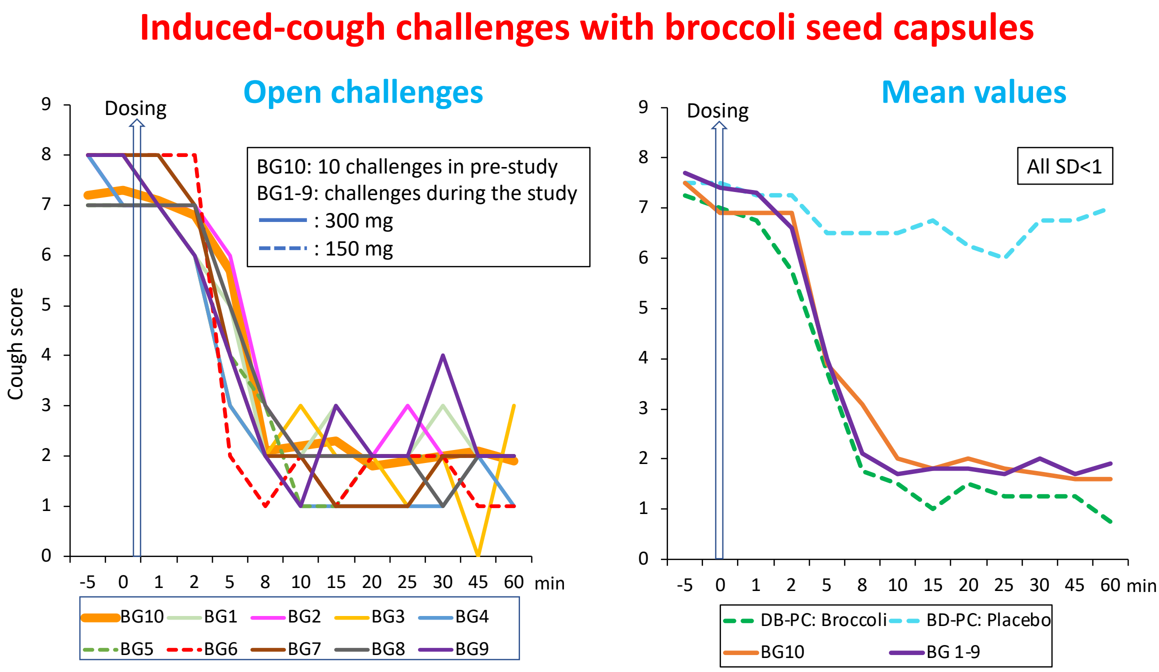
The study was not double-blind since (i) the doses of some nutrients were up to 4 times higher than those recommended and there was a need to assess safety, and (ii) depending on the results obtained with the compound, the patient was able to design further challenges. In the induced cough challenges, symptoms were measured 5 minutes before dosing and at 1, 2, 5, 8, 15, 20, 25, 30, 45 and 60 minutes after. In many cases, the test was continued until induced cough scores were ≥7, and induced cough challenges were repeated every hour. It was *a priori* proposed that the cutoff score level was 3/10 to discriminate a positive and a negative test. Induced cough, spontaneous cough and nasal obstruction were recorded throughout the study.

2. Results

2.1. Broccoli challenges

The results of the 9 broccoli challenges were very similar to both the 4 DB-PC broccoli challenges and the 10 open challenges performed before the onset of the study. This indicated that (i) there was a high reproducibility of the challenges and (ii) the patient had retained the same reactivity to induced cough throughout the study (Figure 1). Within 5-10 minutes after dosing, induced cough scores decreased from 7-8 to 1-3 and this was sustained for the following 50 minutes. A lower broccoli dose elicited the same effect.

Figure 1: Induced cough challenges with broccoli seed capsules



2.2. Rapid onset efficacy of Nrf2/TRAP1/TRPV1 nutrients

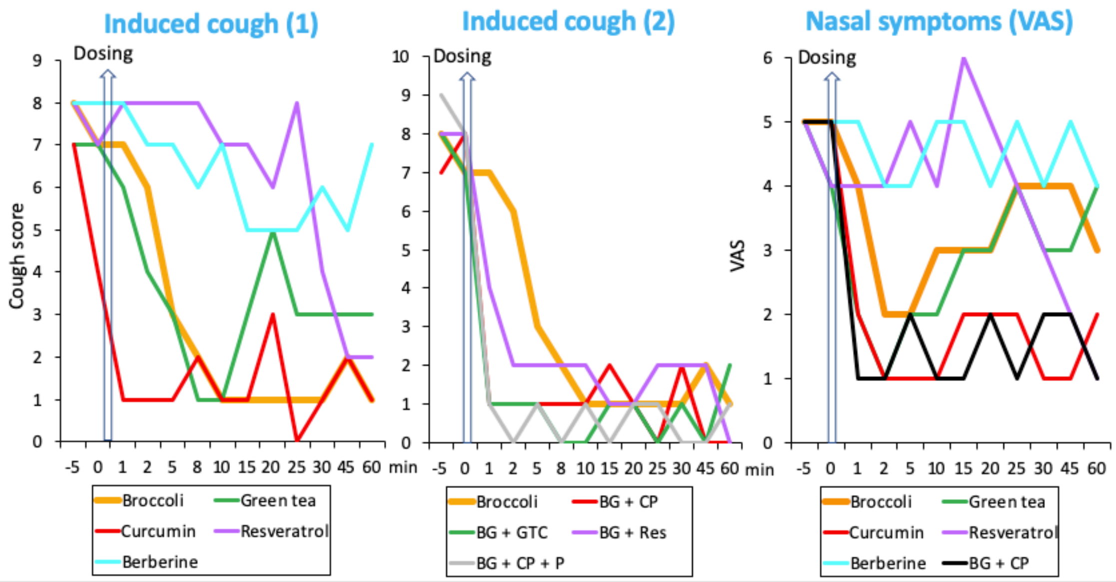
Several nutrients and paracetamol were used in the study (Table 2). Induced cough score was severe (score ≥7/10) in all challenges before dosing. There was no effect for low-dose green tea (N=2), high dose berberine (N=2) or regular dose zinc (N=2). A significant effect (score ≤ 3) was observed within 2 minutes for curcumin + black pepper (N=2), ginsenc (N=2); red pepper (N=3); 8-10 minutes for broccoli (N=9) and high-dose green tea (N=2), 20 min for paracetamol (N=1) and 30-45 minutes for resveratrol (N=3) (Figure 2, panel 1 and Figure 3).

When low-dose broccoli (150 mg) was combined with low or regular doses of green tea capsules (N=2), half dose of ginger (N=2), half doses of curcumin + black pepper (N=2), or half doses of red pepper red pepper (N=2), the effect on cough began one minute after dosing and VAS was below 3 in 2 minutes. When combined with low-dose resveratrol, the effect on cough began at 2 minutes (N=5) (Figure 2, panel 2).

For nasal symptoms assessed before the cough challenges (Figure 2, panel 3), the effect (nasal VAS ≤ 2) was observed within 1 minute for curcumin + pepper and higher doses of green tea, 2-5 minutes for broccoli, 25 min for paracetamol and 30-45 minutes for resveratrol. There was little effect for lower doses of green tea or zinc and no effect for berberine. All combinations - except those with green tea capsules - were rapidly effective. The two challenges with broccoli + green tea capsules were only followed by a small reduction in nasal symptoms (VAS 3) for the first 45 minutes.

Figure 2: Open labelled induced cough challenges with Nrf2/TRPA1 interacting nutrients

The best example for each nutrient is shown



2.3. Impact of Nrf2/TRAP1/TRPV1 nutrients during the first hour after dosing

The induced cough total score for each compound was compared with the mean values of the pre-study challenges carried out with 10 broccoli capsules (300 mg) (Figure 3). The 9 broccoli challenges showed similar results for 300 and 150 mg. On the other hand, the scores were higher for green tea but, more importantly, for berberine, paracetamol, resveratrol and zinc. Conversely, the scores were far lower for curcumin + black pepper, ginger, red pepper and all combinations including those with resveratrol.

Results differed for spontaneous cough occurring between challenges. Broccoli capsules had the lowest scores followed by curcumin + black pepper, most combinations, green tea, berberine and resveratrol.

Figure 3: Global results

	Product	Dose	Active metabolite (dose)	Mechanism	Induced cough challenge				Side effects (VAS)
					Induced cough 0-60	Spont. cough 0-60	Nasal VAS 0-60	Duration (min)	
	Broccoli (N=10)*	300 mg	Glucoraphanine 30 mg Kaemferol	Nrf2>>TRP [34-36]	32				
1	Broccoli				38	4	34		
2	Broccoli				38	4	35		
3	Broccoli				30	5	25		
4	Broccoli				27	5	26	420	
5	Broccoli				32	4	28		
6	Broccoli				35	8	31	300	
7	Broccoli				36	7	28		
8	Broccoli	150mg			36	4	42	360	

9	Broccoli		Glucoraphanine 15 mg		32	4	45		
10	Berberine	1.2 g		Nrf2>TRP [37,38]	63	33	29	60	
11	Berberine	4.8 g			76	43	45	60	
12	Curcuma + black pepper	103 mg 16 mg	Curcumin Piperine	Nrf2-TRPA1 curcuma [18,34,39,40]	17	4	15	240	HB: 2
13	Curcuma + black pepper	51 mg 8 mg	Curcumin Piperine	peper[41-43]	26	10	32	90	HB: 2
14	Ginger 2 caps	300 mg	Gingerol 12.6 mg	Nrf2-TRP[44-46]	15	5	13	240	
15	Green tea bag	3 g		Nrf2>TRP[19,20]	54	12	34		
16		4.5 g			35	13	31		
17	Green tea capsule	1.75 g			47	17	27	60	
18	Green tea loose	unknown			54	12	33		
19	Quercetin	2 g		Nrf2>TRP[34,36,47]	78	21	53	60	
20	Red pepper	10 mg	Capsaicin	Nrf2<<TRPV1[41,48]	21	11	18	120	HB: 2
21		20 mg		42,43	17	4	22	180	HB: 3
		30 mg			14	4	19	300	HB: 4
23	Resveratrol	600 mg		Nrf2>TRP[34,38,49]	63	14	46		
24		600 mg			68	32	38	300	
25		1200 mg			68	42	44	360	
26	Zinc	15 mg		Nrf2=TRP[22,23,50]	67	34	46	60	HB: 2
26	Paracetamol metabolites	500 mg		TRPA1-TRPV1[24,27]	53	34	37	180	HB: 2
27	BG 150 + CU 50 + BP 8				13	6	14	500	HB: 2
28	BG 150 + CU 50 + BP 8				10	4	15	440	HB: 3
29	BG 150 + Ginger 150				19	3	14		
30	BG 150 + Res 300				20	7	19	600	
31	BG 300 + Res 300				20	11	22	600	
32	BG 300 + Res 300				16	12	26		
33	BG 150 + GTC 1700				22	3	24		
34	BG 150 + GTC 3250				8	14	29	600	
35	BG 150 + CU 50 + BP 8 + RP 20				5	6	11	780	HB: 4
36	BG 150 + CU 50 + BP 8 + paracetamol 250				6	7	18	900	HB: 2
37	BG 150 + CU 50 + BP 8 + paracetamol 250				11	2	16	720	
38	BG 150 + RP 20 + paracetamol 250				5	4	12	1020	HB: 6 GIP: 2*
39	BG 150 + Ginger 150 + paracetamol 250				11	2	13	720	HB: 3

Spont.: Spontaneous, HB: Heartburn, GIP: Intestinal pain, *: need for lansoprazole
BG: Broccoli, CU: curcumin, BP: Black pepper, Res: Resveratrol, GTC: Green tea capsule, RP: Red pepper
From 27 to 39: doses are in mg

Induced cough score (0-10*)	<20	20 to 29	30 to 39	40 to 59	≥59
Spontaneous cough score (0-10*)	<5	5 to 9	10 to 19	20 to 39	≥40
Nasal VAS (0-10*)	<15	15 to 19	20 to 29	30 to 39	≥40
Duration (min)	>660	421 to 660	181 to 420	60 to 180	≤60
*: 0: best, 10: worst					

Nasal symptoms were assessed before the cough challenge. Nasal total score (VAS) was similar for broccoli 300 mg, green tea and low-dose curcumin + pepper. It was lower for curcumin + pepper high dose and for all combinations. It was higher for broccoli 150 mg, berberine, resveratrol, zinc or paracetamol. A dose-response relationship was found for curcumin + black pepper or red pepper in nasal symptoms. There was a dose-dependent effect for broccoli (150 and 300 mg), curcumin + black peper and red pepper.

2.4. Duration of the benefits of Nrf2/TRAP1 nutrients

In 26 cough challenges, a follow up was carried out until the patient experienced spontaneous cough (VAS≥7) or a cough score ≥7 during the challenge (Figure 3). The duration of efficacy was ranging from 300 to 420 minutes in the broccoli challenges, whatever the dose ingested. It was lower for green tea, resveratrol and particularly in curcumin + pepper, red pepper or paracetamol with a dose-dependent effect.

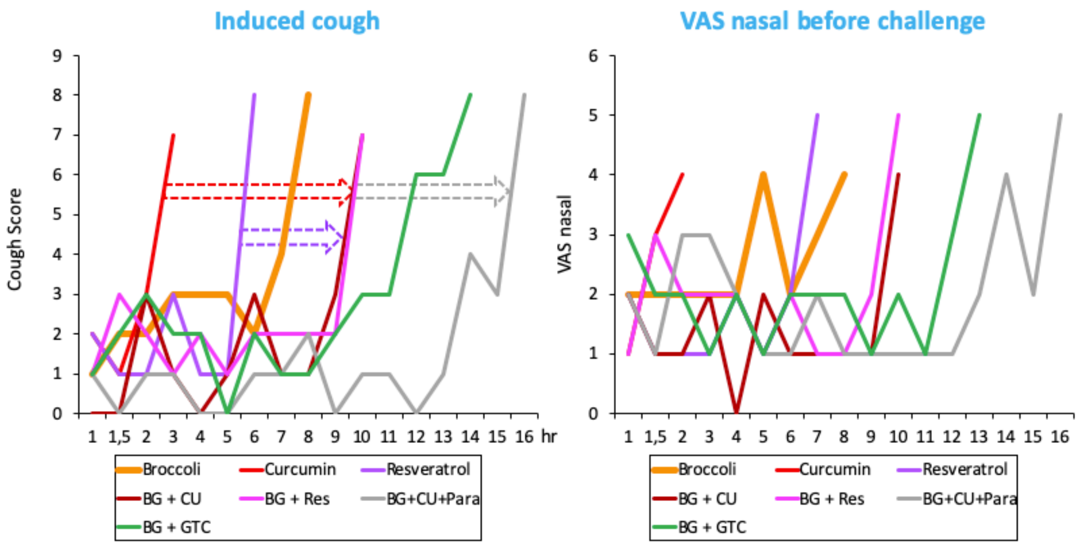
For all broccoli combinations without paracetamol, the duration of action ranged from 440 to 600 minutes except for the combination with red pepper (780 minutes).

For all broccoli combinations with paracetamol the duration of action ranged from 720 to 1020 minutes.

2-5- Symptoms experienced after the first hour

Representative examples of symptoms experienced after the first hour are presented in Figure 4.

Figure 4: Symptoms experienced after the first hour post challenge



The arrows indicate the increased duration of adding broccoli to curcumin (red) or resveratrol (purple), and adding paracetamol to broccoli and curcumin (grey).

Induced cough was relatively well controlled (score ranging from 0 to 2 or 3/10) until the patient had a severe score with rapid onset. Nasal symptoms were similar to induced cough and spontaneously increased when the patient lost control of the disease.

During all of these challenges, there were some rapid, transient (less than one to 3 minutes) and mild episodes of tracheal irritation followed by one or two cough episodes and some nasal obstruction. These episodes were relatively common within the first hour following the ingestion of the compounds. At the end of the test, when the compound was becoming less effective, these waves were increasing in frequency and severity and were associated with increasing cough and nasal obstruction.

When the patient initially took capsules containing curcumin and black pepper, he experienced some gastro-esophageal discomfort and heartburn (VAS 2-3/10 for pain). However, when red pepper was associated with paracetamol heartburn increased (VAS 6) and required lansoprazole 30 mg. This discomfort lasted for around one to 2 hours.

3- Discussion

3-1- Strengths and limitations

All the challenges were carried out on the same patient (author of the paper). The cough challenge is highly reproducible as shown for broccoli (10 open labelled in the pre-study, 4 DB-PC and 9 open labelled in the study). Moreover, it is very easy to count cough episodes. Nasal VAS may be less easy to assess but the patient has a much expertise on it.

One single patient was used for the challenges which has both strengths and weaknesses.

The choice of the nutrients was not easy but we tried to find Nrf2, TRPA1 and TRPV1 agonists as clear cut as possible. One important TRPA1 agonist is allicin that was not used since the patient is intolerant to garlic. Mustard oil alone cannot be found in a nutrient in France. Only a few challenges were carried out with the same nutrient but we wanted to study a larger number. The quality of the products raises other questions. The broccoli capsule was studied.

We do not have the exact composition of each nutrient and we used the labelled one.

The cough challenge does not reflect real life since inducing cough activates TRAP1/TRPV1 receptors that are sensitive to mechanical stress in a way that is not encountered in real life.

3-2- Optimal combination to be further tested in clinical trials

The results of this study are indicative of the efficacy of Nrf2 and TRPA1/TRPV1 agonists, but the best combination may not have been tested. The exact composition of each nutrient is required and its overall oxidative potency needs assessment. Then, different mixtures can be tested in controlled clinical trials. However, the results of the present study indicate that Nrf2 agonists or TRPA1/TRPV1 agonists alone are insufficient in the control of cough and nasal symptoms following COVID-19 infection. The combination was more effective and lasted for a longer period of time.

3-3- Possible putative mechanisms

Possible mechanisms underlying these results are presented in Table 3. They however need to be confirmed well-designed studies.

Table 3: Interpretation of the results

	Effect observed	Tentative interpretation
1	All effective nutrients (except resveratrol and paracetamol) acting on induced cough and nasal symptoms are effective between 1 to 10 minutes.	This suggests a neurologic mechanism. In all conditions associated with COVID-19, there is an impaired redox homeostasis responsible for reactive oxygen species accumulation [1]. TRPA1 is highly sensitive to oxidative stress [51]. Several COVID-19 symptoms may be induced by TRPA1/TRPV1 activation [52]. TRPA1 may be activated during COVID-

		19 and can be down-regulated very rapidly, possibly explaining the extremely swift onset of nutrients [52]. Paracetamol was effective after 20 minutes since only its metabolites are TRPA1/TRPV1 agonists. [24,25]
2	Broccoli capsules that are effective on some symptoms of COVID-19 reduce nasal symptoms and induced cough from 5-10 minutes to around 4-6 hours.	Broccoli contains • relatively low levels of TRPA1/TRPV1 explaining its moderate effects by comparison to other nutrients for the first hour; • glucoraphanin and myrosinase that can produce sulforaphane [6] within 30 minutes [53], the most potent Nrf2 natural agonist [54], possibly explaining the slightly delayed and long-term effects.
3	Berberine, an Nrf2 agonist that does not have an apparent TRPA1 or TRPV1 interaction, is not effective on nasal symptoms and induced cough.	A moderate Nrf2 activity may be insufficient for the control of COVID-19 symptoms.
4	Zinc, at the recommended dose of 15 mg, is ineffective either as a single challenge (N=1) or in combination with broccoli and curcumin + green pepper (N=1)	The dose of Zinc may be too low.
5	Curcumin and black pepper reduce nasal symptoms and induced cough more rapidly, but their effect is not sustained after 2-3 hours.	Curcumin is a TRPA1 agonist [18] and black pepper a TRPA1 and TRV1 agonist [55] (and both are Nrf2 agonists). Their potent and rapid effect is probably associated with the desensitization of the receptors.
6	There is a dose-dependent effect of curcumin and black pepper or red pepper. At the daily recommended dose, the capsules are more effective on nasal symptoms and induced cough than when using half a dose.	This is in line with receptor desensitization.
7	High doses of green tea reduce nasal symptoms and induced cough rapidly and for a short period (< 1 hr).	Polyphenols of green tea are TRPA1 agonists [19,20] (and Nrf2 agonists).
8	Resveratrol induces a delayed (30-45 min) reduction of nasal symptoms and induced cough.	Resveratrol is another TRPA1 agonist (and Nrf2 agonists) but its mechanisms of action may need some time to be effective [21,56].
9	When broccoli (at half dose) is added to low-dose curcumin + black pepper, ginger or red pepper, high-dose green tea or regular dose resveratrol, the effects are very rapid and last for over 8 hrs.	The prolongation of the effects of both broccoli (sulforaphane) and TRAP1/TRPV1 agonists suggests an interaction between the two mechanisms. It is likely that TRAP1 desensitization is prolonged by the Nrf2 anti-oxidant effects of sulforaphane, leading to a down-regulation of the receptor.
10	The addition of paracetamol (low dose) to broccoli and curcumin + black pepper, red pepper prolongs the effects of the combination to over 14 hrs.	Some paracetamol metabolites (N-acetyl-p-benzoquinone imine) are TRPA1/TRPV1 agonists [24]. During the cytokine storm in three patients, it was found that broccoli effects increased from around 6 to 10-12 hrs by paracetamol [9]. The prolongation of the effect reproduced in the current challenges is likely to be associated with TRAP1, since paracetamol takes some time to be metabolized.
11	Spontaneous cough is increased with the TRAP1/TRPV1 agonists by comparison to broccoli alone. These cough episodes (usually one or two	These episodes probably reflected the transient activation of the TRPA1/TRPV1 receptors followed by immediate desensitization.

	mild coughs) occurred simultaneously with nasal obstruction and irritation of the larynx and trachea for less than one minute. They had no impact on the results of the following spontaneous challenge.	
12	At the end of the beneficial effect of the Nrf2 or TRPA1/TRPV1 -interacting nutrients, cough, nasal obstruction and tracheobronchial irritation increased very often simultaneously.	These findings suggest that TRPA1/TRPV1 receptors were active again.
13	At the end of the beneficial effect of the nutrients, a gastroesophageal discomfort occurred only with curcumin and black pepper. This effect lasted for 2-3 hrs and was very similar to symptoms occurring after wasabi ingestion when the patient ate sushi [57].	Wasabi is a potent TRPA1 agonist [57]. These findings suggest the reactivation of TRPA1 receptors. There may be an interaction with TRVP1 receptors. The findings of 11-13 strongly suggest TRPA1/TRPV1 desensitization.

3-4- Implications

In the current study, the short duration of the effect of green tea or curcumin + black pepper or resveratrol suggests that TRP desensitization does not last long with the doses ingested. This is very important since, in Westernized diet, the amount of spices regularly consumed is usually low.

Desensitization of TRPV1 underlies the paradoxical analgesic effect of capsaicin. The TRPV1 receptors begin a refractory state, commonly termed as desensitization, that leads to the inhibition of receptor function [58]. The acute desensitization of TRVP1 accounts for most of the reduction in responsiveness occurring within the first few (~20) seconds after the vanilloids are administered to the cell for the first time. Several signalling pathways including calcineurin, calmodulin or the decrease of Phosphatidylinositol 4,5-bisphosphate (PIP_2) [59] are involved in TRPV1 desensitization. TRPA1 is desensitized by homologous (mustard oil; a TRPA1 agonist) or heterologous (capsaicin; a TRPV1 agonist) agonists via Ca2+-independent and Ca2+-dependent pathways in the sensory neurons [60]. These rapid desensitization processes are likely to be involved in the rapid effects of TRPA1/V1 nutrients found in the current study.

Another form of TRP desensitization is 'tachyphylaxis', which is a reduction or the disappearance in the response to repeated applications of agonists [61-63]. In Asian or Sub-Saharan countries, large amounts of spices are regularly eaten and it is likely that TRPA1/V1 receptors are permanently desensitized or have disappeared allowing people to eat large amounts. It is therefore possible that TRPA1/V1 tachyphylaxis may reduce the severity of COVID-19 symptoms, and even possibly reduce infection by SARS-CoV-2.

The increased duration of the effect when lower doses of curcumin + black pepper or red pepper were added to broccoli suggests that oxidative stress down-regulated by sulforaphane allows the desensitization of TRPs for a longer period of time. Moreover, for the first hour after ingestion, small waves of tracheal irritation, cough and nasal obstruction were perceived, suggesting activation-deactivation of the receptors. It probably takes one hour for sulforaphane to be synthesized and active in reducing the oxidative stress. This would indicate a cross talk between the anti-oxidative effect of Nrf2 and the desensitization of TRPA1/V1. This information is critical as it allows to propose low doses of TPRA1/V1 agonists that will be potentiated by Nrf2 agonists in COVID-19. The doses of agonists is still unclear and needs to be studied further.

Research should confirm these data and the intertwined mechanism of great importance in order to develop medications and foods based on TRPA1, TRPV1 and Nrf2 agonists. Moreover, more effective nutrients can be proposed.

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Abbreviations

Broccoli: Broccoli seeds containing 10% glucoraphanin
COVID-19: Coronavirus 19 disease
ER: Endoplasmic reticulum
NAPQI: N-acetyl-p-benzoquinone imine
Nrf2: Nuclear factor (erythroid-derived 2)-like 2
ROS: Reactive oxygen species
SARS: Severe acute respiratory syndrome
SARS-Cov-2: Severe acute respiratory syndrome coronavirus 2
TRP: Transient receptor potential
TRPA1: Transient receptor potential ankyrin 1
TRPV1: Transient receptor potential vanilloid 1